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TRANSACTIONS

Studies on Vitamin B_2 Complex.

II—Some Observations on the Nutritive Value of Sugars.*

By Ume TANGE and Tetsuko KANEKO.

(From the Institute of Physical and Chemical Reserch.)

Received Oct. 23, 1938.

Previously one⁽¹⁾ of us (U. T.) demonstrated that when rats were fed on sucrose diet they developed the pellagra-like symptoms and failed to grow, but this condition was protected and cured by acid clay adsorbate or its eluate of rice polishings extracts, and described that the factor might be identical with vitamin B_6 described by György.

Birch, György and Harris⁽²⁾ showed that cane molasses is very rich in vitamin B_6. Furthermore, Jukes and Lepkovsky⁽³⁾ recently found in cane molasses a fairly good source of the preventive factor, the so-called filtrate factor, for chick dermatitis.

In nutrition as well as in domestic economy molasses is, therefore, more desirable to be used in daily life than pure white sugar, though molasses is still generally regarded as a qualitatively inert food constituent. It is very interesting to note that in ancient times "Kurozato" (a crude brown coloured sugar containing molasses) was commonly used by Japanese, whereas pure white sugar was used for medicine or only by rich people as precious food, and also that polished rice had been substituted for half-polished rice or "Haigamai" (rice with germ) which is more nutritious than the former.

The present paper contains the results of experiments on the nutritive value of "Kurozato", "Ganmitsuto" (a brown coloured sugar containing molasses), exhausted cane molasses, and sucrose.

EXPERIMENTAL.

Part I. (*Preliminary experiment*).

The methods employed in the present work were similar to those described in the previous paper.⁽¹⁾ When the weight of rats had remained stationary constant for several days, crystalline vitamin B_1 hydrochloride was supplied daily at the level of 10 γ.

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* This paper was presented at the Scientific Meeting of the Institute of Physical and Chemical Research, December 16, 1937.

The basal rations had the following composition : †

Rations Component	Sucrose	"Kurozato"	"Ganmitsuto"	Cane molasses
Purified fish protein	20	20	20	20
Crisco	3	3	3	3
McCollum's salt mixture	5	5	5	5
Dried egg white powder (as flavin)	5	5	5	5
Agar-agar	2	2	2	2
Sucrose (*pharmacopœia Japonica*)	65	—	—	—
"Kurozato" (commercial)	—	65	—	—
*"Ganmitsuto"	50	—	15	—
*Exhausted cane molasses	60	—	—	5

† In addition to the diets all animals received 1 drop of cod liver oil daily for vitamins A and D.

* "Ganmitsuto" and molasses were provided by Meiji-Seito Company. We wish to thank them for the generous contribution.

Analysis of Samples (%).

	"Ganmitsuto"	"Kurozato"	Exhausted cane molasses
Moisture	1.94	7.16	22.82
Solid	98.06	92.84	77.18
Sucrose	86.83	79.72	34.79
Invertose	3.22	8.00	17.72
Ash	5.94	3.59	14.13
Total nitrogen	0.03	0.14	0.42
Organic matter	2.04	1.39	10.12
	100.00	100.00	100.00

Components of Ash.

	"Ganmitsuto"	"Kurozato"	Exhausted cane molasses
Fe_2O_3	12.75	8.60	2.64
CaO	27.30	25.08	23.98
K_2O	36.17	22.80	45.25
P_2O_5	1.35	7.75	5.20
Insoluble portion	5.93	27.24	18.16
Remainder	16.50	8.53	4.77
	100.00	100.00	100.00

The results are summarized as follows:

1. The rats fed on the diet composed of 65 per cent sucrose as source of carbohydrate, supplemented daily with $10\,\gamma$ vitamin B_1, developed pellagra-like symptoms and died within 6 to 8 weeks, while on "Kurozato" diet containing as high percentage as sucrose, the animals showed none of the symptoms, but attained normal growth, if they were administered daily with $10\,\gamma$ vitamin B_1.

2. If 5 per cent of sucrose diet was replaced by the equal amount of molasses, the animals attained as good condition as those on "Kurozato" diet.

3. On the diet composed of 50 per cent sucrose plus 15 per cent "Ganmitsuto", the rats showed as healthy appearance as those on the latter two diets.

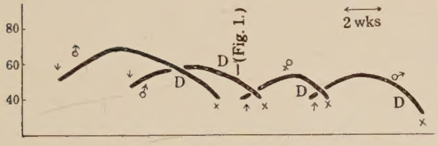

Chart 1. Growth curves of rats fed on sucrose diet (65%).

↓ or ↑ ······B_1. D······dermatitis.

×······death.

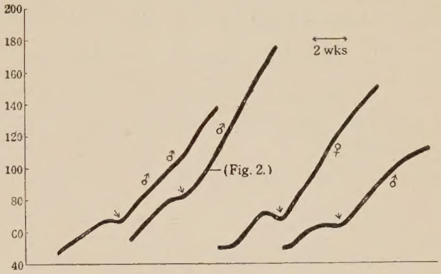

Chart 2. Growth curves of rats fed on "Kurozato" diet (65%).

↓······B_1.

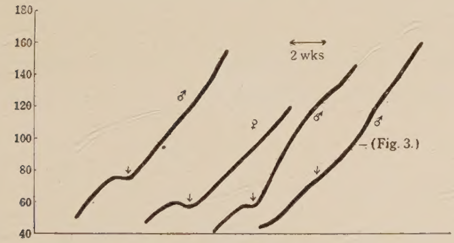

Chart 3. Growth curves of rats on molasses diet (60% sucrose + 5% molasses).

↓······B_1.

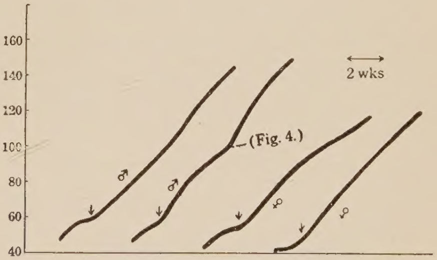

Chart 4. Growth curves of rats on "Ganmitsuto" diet (50% sucrose + 15% "Ganmitsuto").

↓······B_1.

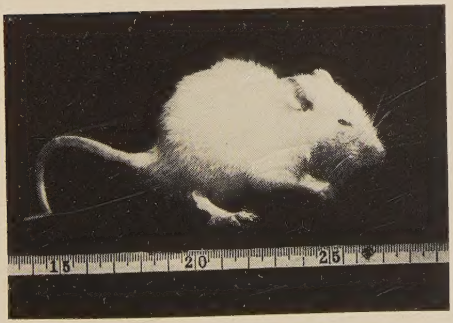

Fig. 1. Showing the dermatitis resulting from sucrose (65%) as the basal carbohydrate.

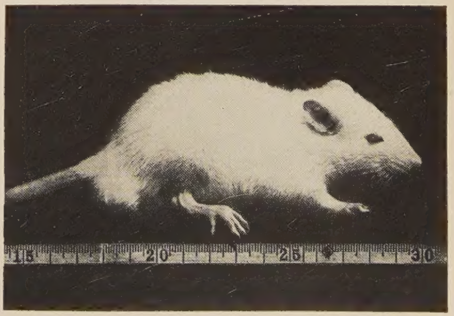

Fig. 2. Showing the healthy condition of the rat fed on "Kurozato" diet (65%).

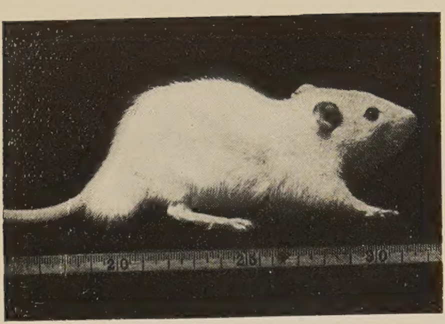

Fig. 3. Showing the normal growth of the rat fed on "Ganmitsuto" diet (Sucrose 50+ "Ganmitsuto" 15%).

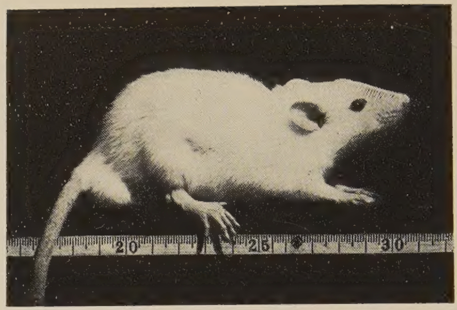

Fig. 4. Showing the normal growth of the rat on molasses diet (Sucrose 60+ Molasses 5%).

These rats were litter mates. They were photographed on the thirty-ninth day of experiment, at which they weighed 52 g (Fig. 1), 98 g (Fig. 2), 97 g (Fig. 3), and 100 g (Fig. 4), respectively.

Part II.

In the preliminary experiment (*Part I*) we found that, in the rations mentioned above, if "Kurozato", "Ganmitsuto", and molasses were totally or partially substituted for sucrose, the animals on these diets did not develop the pellagra-like symptoms as in the case of sucrose diet, but attained normal growth.

Recently György(4) reported that crude cane molasses is good source of vitamin B_6 and also contains small amounts of flavin,

In this experiment we tried to find if the preceding crude sugars and molasses contain flavin, and also to separate the active factors essential for the nutrition of rats.

METHODS.

The growth of rats and the protection of the characteristic dermatitis of the dietary deficieney were employed in the assay of the fractions. The animals were placed on experiment in groups of from 4 to 6 animals each, care being taken in their group distribution in order to minimize both litter and sex variations.

The basal rations used in this experiment were similar to those described in *Part I*, but free from flavin, and had the following composition: Purified fish protein 20, Sugars 65, Crisco 3, Agar-agar 2, and McCollum's salt mixture 5, provided with 1 drop cod liver oil daily.

The rats received the rations deprived of flavin, had usually ceased to grow after two weeks, even though they were administered daily with 10 γ of vitamin B_1.

Preparation of supplementary materials—Separation of the active fractions from the crude sugars and molasses was carried out according to the method described in the previous publication.(1) The materials were exhausted carefully with 88~90% alcohol several times to remove inert substances. The combined alcohol extracts were then concentrated in vacuo to a suitable volume. One part of this concentrate was stored for assay, the other was diluted with H_2O, acidified with

H_2SO_4 to PH 2.0, and then adsorbed on acid clay repeating three times. The adsorbates were combined and eluted by shaking with 0.2 *N* $Ba(OH)_2$, and Ba was removed with H_2SO_4. The final filtrate from acid earth adsorbate was treated with $Ba(OH)_2$ to remove H_2SO_4. Both the eluate and filtrate were stored for test.

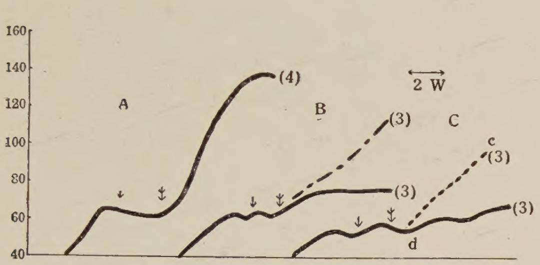

Chart 5. Growth curves of rats on sucrose plus molasses ration, and sucrose ration supplemented with the fractions from molasses. A······60% sucrose +5% molasses. B······65% sucrose and eluate of acid earth adsorbate (=5 g molasses); — — — — — added acid earth filtrate (=5 g molasses). C······65% sucrose and acid earth filtrate (=5 g molasses); — — — — added eluate of acid adsorbate (=5 g molasses).

↓······B_1. ⇟······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

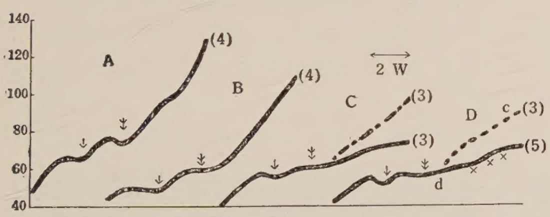

Chart 6. Growth curves of rats on "Kurozato" ration, and sucrose ration supplemented with the fractions from "Kurozato". A······65% "Kurozato". B······65% sucrose and concentrate of alcohol extract (=5 g "Kurozato"). C······65% sucrose and eluate of acid earth adsorbate (=5 g "Kurozato"); — — — — — added acid earth filtrate (=5 g "Kurozato"). D······65 % sucrose and acid earth filtrate (=5 g "Kurozato"); — — — — added eluate of acid earth adsorbate (=5 g "Kurozato").

↓······B_1. ⇟······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

RESULTS AND CONCLUSIONS.

The results with various sugars and their fractions with or without flavin were shown in Charts 5 to 7. Flavin was provided as dried egg white powder at the level of 6 per cent.

The results showed that "Kurozato", "Ganmitsuto", and cane molasses contained at least two factors essential for the rat growth. One factor, resembling vitamin B_6, was adsorbed on acid clay from the extracts of the sugars, the other was present in the filtrate after exhaustive treatment with acid clay. These sugars, however, did not contain vitamin B_1 and flavin as seen from the growth curves. We previously reported(1) that the vitamin B_2 requirement of rats was satisfied only when flavin, B_6 and an acid earth filtrate from liver extract were supplied together.

There is, at present, ample evidence that rats require for maximum growth four distinct factors of the vitamin B complex, namely, vitamin B_1, riboflavin, vitamin B_6, and the so-called filtrate factor.

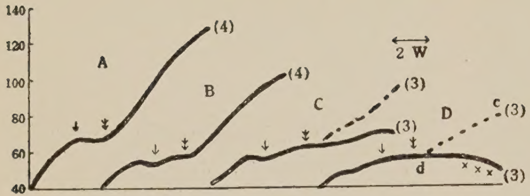

Chart 7. Growth curves of rats on sucrose plus "Ganmitsuto" ration, and sucrose ration supplemented with the fractions from "Ganmitsuto". A······50% sucrose +15% "Ganmitsuto". B······65% sucrose and concentrate of alcohol extract (=5 g "Ganmitsuto"). C······65% sucrose and eluate of acid earth adsorbate (=5 g "Ganmitsuto"); — - — - — added acid earth filtrate (=5 g "Ganmitsuto"). D······65% sucrose and acid earth filtrate (=5 g "Ganmitsuto"); — — — added eluate of acid earth adsorbate (=5 g "Ganmitsuto").

↓······B_1. ↡······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

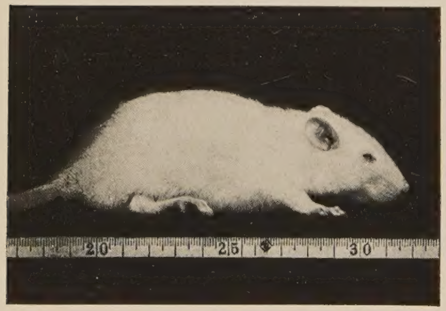

Fig. 5. Showing the influence of the concentrate of alcohol extract of "Kurozato" on the growth (body weight 97 g).

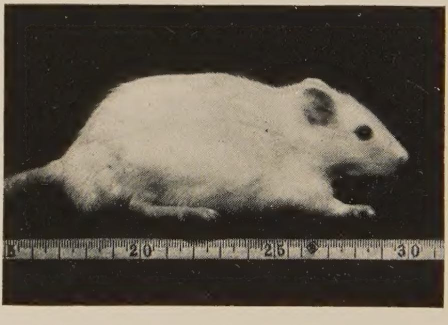

Fig. 6. Showing the influence of the concentrate of alcohol extract of "Ganmitsuto" on growth (body weight 102 g).

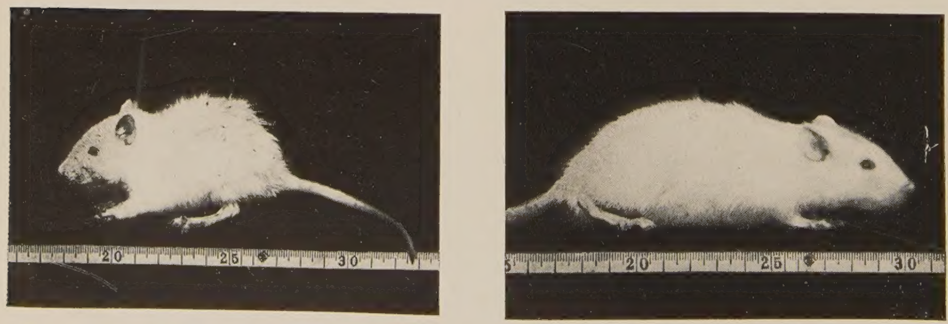

Fig. 7. (a)—Showing the inability of acid earth filtrate of cane molasses on preventing dermatitis caused by high sucrose diet (body weight 52 g).

(b)—Showing recovery of the rat (a) by supplementing eluate of acid earth adsorbate from molasses extract (body weight 88 g).

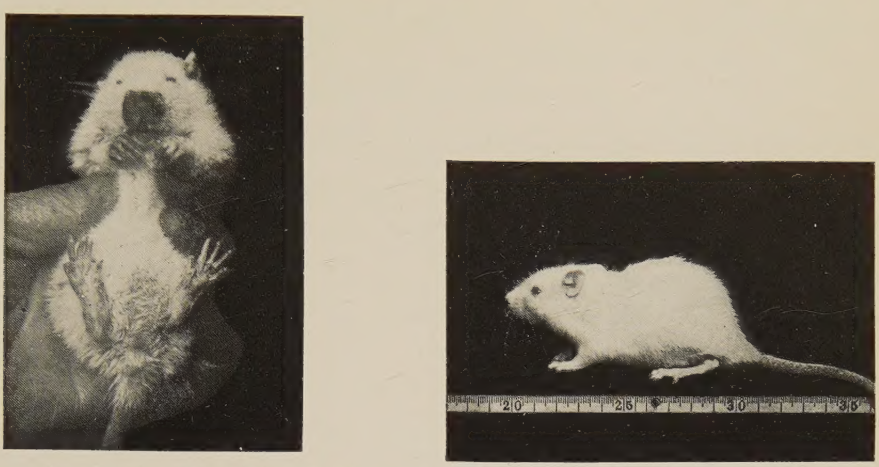

Fig. 8. (a)—Showing the inability of acid earth filtrate of "Ganmitsuto" to protect dermatitis due to high sucrose diet (body weight 50 g).

(b)—Showing recovery of the rats (a) by adding of eluate of acid earth adsorbate from "Ganmitsuto" extract (body weight 68 g).

SUMMARY.

1. When sucrose was used at the basal carbohydrate, the rats developed dermatitis, whereas if sucrose was totally or partially substituted by "Kurozato", "Ganmitsuto", or cane molasses, no dermatitis resulted.

2. The concentrate of alcohol extracts of the crude sugars mentioned above cured the dermatitis occurring in the rats fed on sucrose diet and promoted growth, provided adequate amounts of vitamin B_1 and flavin were fed simultaneously.

3. The data indicated that the concentrate of the crude sugars contained at least two factors essential for the nutrition of rats. One factor was adsorbed on acid clay, from which was eluted with 0.2 N $Ba(OH)_2$, the other was present in the acid earth filtrate; and the former may be identical with vitamin B_6 and the latter resembles the so-called filtrate factor.

We wish to thank Dr. U. Suzuki for his deep interest and helpful advice throughout this work. We are also indebted to Dr. T. Tanno for the analysis of samples.

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Isolierung einer für die Vergärung des Zuckerrohrsaftes geeigneten Hefe aus dem Verdauungsorgan des Grashopfers.

Von Kano OHTSU und Kazuo YAMAFUJI.

(Aus dem Institut für Zuckerforschung in Tainan.)

Eingegangen am 9, Feb. 1939.

Gewöhnlich enthält der Verdauungssaft des Insekts verschiedene Mikroben. Die Enzyme dieser Mikroorganismen sind in einigen Fällen an den Verdauungsvorgängen beim Insekt beteiligt. In den anderen Fällen wird aber die Vitalität des Insekts durch das Toxin der Bakterien oder Pilze im Digestionssaft geschädigt. Wir haben im Zuckerrohrfeld einige Grashopfer gesammelt und das Gärvermögen der aus ihren Verdauungsorganen isolierten Hefen untersucht.

1. Isolierung der Hefen.

Nachdem man den Grashopfer mit 80% igem Alkohol desinfiziert hatte, wurde ihm das Verdauungsorgan entnommen. Von dem Inhalt dieses Organs brachten wir mittels einer kleinen sterilen Platinöse eine geringe Menge in den verflüssigten Kojiagar-Nährboden. Aus diesem Nährboden wurden in der gewöhnlichen Weise eine Reihe von Plattenkulturen hergestellt. Wir führten dann die Reinzucht der Hefe nach dem *Lindner*schen Tröpfchenverfahren durch. Weiter wurde die Hefe wiederholt auf dem Zuckerrohrsaft-Agar gezüchtet.

2. Ermittelung der Gärtätigkeit der isolierten Hefen.

Durch vorläufige Gärversuche wurden von den 18 Hefenarten, welche man in der oben angegebenen Weise aus dem Verdauungssaft des Grashopfers als Reinkulturen erhielt, zunächst 4 Sorten gewählt. Nachdem wurde dann die Gärfähigkeit dieser 4 Arten Hefen eingehender untersucht. Zu dieser Erforschung wurden die Hefen von der fünftägigen Zuckerrohrsaft-Agarkultur mit Hilfe eines Platindrahts in 50 ccm Saft aus dem 13 Monate alten Zuckerrohr gezüchtet. Den Gärverlauf stellten wir nach der früher mitgeteilten Methode (1) (2) fest.

Tabelle I. Eigenschaften des Saftes vor dem Vergären.

Grad Balling	12.26	Gesamtstickstoff in %	0.043
Reduzierender Zucker in %	1.10	Asche in %	0.23
Rohrzucker in %	8.61	pH	4.9
Gesamtzucker in %	10.15	Azidität	0.3

Azidität bedeutet ccm *n* NaOH pro 100 ccm Nährlösung.

Tabelle II. Gärverlauf.

Art der Hefe	Nr. 1	Nr. 2	Nr. 3	Nr. 4
Gesamtgärzeit in Stdn.	96	96	120	120
Entstandener Alkohol in Volum-%	6.00	6.08	4 54	5.31
Entwickelte Kohlensäure in g	2.32	2.39	1.75	1.98
Endvergärungsgrad in %	91.75	92.96	69.41	81.19

Aus der Tabelle II geht hervor, dass die Hefe Nr. 2 die stärkste Gärwirkung hat. Wir nennen diese Hefe vorläufig „Saccharomyces batta“.

3. Eigenschaften von „Saccharomyces batta“.

a) *Zellform und Zellgröße.* Die im Zuckerrohrsaft von 12° Bg gezüchtete Hefe ist kreisförmig oder ellipsoidisch. Der Durchmesser dieser Hefe beträgt 3.5~5.5 μ.

b) *Riesenkolonie.* Bei der Zucht von Saccharomyces batta auf der Oberfläche des Zuckerrohrsaft-Agars entsteht eine Riesenkolonie, welche einen hervorragenden Kern und einen unebenen Rand besitzt.

c) *Zuckerrohrsaft-Kultur.* Während der Vergärung der Zuckerarten im Zuckerrohrsaft bleibt der Saft trübe. Nach der Gärung aber wird die Nährlösung klar.

d) *Sporenbildung.* Die vorher im Zuckerrohrsaft gewachsene Hefe bildet nach 20 Stunden bei 33° auf dem Gipsblock ihre Sporen aus.

e) *Temperatur.* Bei etwa 33° zeigt die Hefe die stärkste Gärtätigkeit sowie Vermehrungsfähigkeit. Die Tötungstemperatur der Hefe liegt bei 57°.

f) *Wasserstoffionenkonzentration.* Die Hefe vermehrt sich am besten bei pH 3.5~5.6.

4. Gärversuche in der Fabrik.

Versuch 1. 12 Tonnen des 18 Monate alten Zuckerrohrs (Rasse: F 108) wurden unter Anrühren mit Wasser ausgepresst. Der erhaltene Saft betrug 120 hl. Der Saft wurde in einem Gärbottich durch Erhitzen auf etwa 100° sterilisiert und dann wurde der Bottich mit Wasser auf 34° abgekühlt. Die Analyse dieses Saftes gibt folgende Resultate (Tabelle III).

Tabelle III.

Grad Balling	14.36	Gesamtstickstoff in %	0.047
Reduzierender Zucker in %	1.60	Asche in %	0.27
Rohrzucker in %	11.00	pH	5.2
Gesamtzucker in %	13.18	Azidität	0.8

Die Überführung der Hefereinkultur aus dem Laboratorium in den Betrieb erfolgte in der folgenden Weise:

Von einer fünftägigen Zuckerrohrsaft-Agarkultur wurde Saccharomyces batta in 50 ccm Zuckerrohrsaft von 15.62° Bg übergeimpft. Nach 48 stündigem Stehen bei 33° wurde die Hefe von neuem in 500 ccm Zuckerrohrsaft im *Pasteur*-Kolben weiter gezüchtet. Der frisch beimpfte Kolben wurde hierauf in den Thermostat von 33° gebracht und nach 27 Stunden wurde die erhaltene Hefesuspension in einen 150 hl fassenden Gärbottich, der mit 120 hl Zuckerrohrsaft beschickt wurde, eingeführt. Für 120 hl Saft wurden 5 *Pasteur*-Kolben verwendet. Dann wurde bisweilen die Lüftung der Würze ausgeführt.

Der Gärverlauf ist in Tabelle IV wiedergegeben.

Tabelle IV.

Saft nach dem Vergären		Gärung	
Grad Balling	−1.5	Gesamtgärzeit in Stdn.	52
Gesamtzucker in %	0.08	Entstandener Alkohol in Volum-%	8.08
pH	3.6	Endvergärungsgrad in %	95.17
Azidität	3.8		

Versuch 2. Aus 10 Tonnen des 13 Monate alten Zuckerrohrs (Rasse: 2725 POJ) erhielten wir durch Pressung 93 hl Saft. Die Eigenschaften dieses Saftes zeigt Tabelle V.

Tabelle V.

Grad Balling	12.73	Gesamtstickstoff in %	0.043
Reduzierender Zucker in %	2.74	Asche in %	0.30
Rohrzucker in %	7.39	pH	5.1
Gesamtzucker in %	10.51	Azidität	1.9

Die Überimpfung der Hefe in den Gärbottich wurde im wesentlichen in der obenerwähnten Weise durchgeführt. Am Ende der Gärung wurde der Saft analysiert (Tabelle VI).

Tabelle VI.

Saft nach dem Vergären		Gärung	
Grad Balling	−1.0	Gesamtgärzeit in Stdn.	46
Gesamtzucker in %	0.138	Entstandener Alkohol in Volum-%	6.44
pH	3.7	Endvergärungsgrad in %	95.13
Azidität	4.3		

Zur Feststellung des Gärvermögens von „Saccharomyces batta“ müssen die Versuche im Fabrikbetrieb noch wiederholt angestellt werden.

Zusammenfassung.

Aus dem Verdauungssaft des Grashopfers im Zuckerrohrfeld wurde eine Art Hefe isoliest, die den Zuckerrohrsaft sehr stark zu vergären vermag.

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Some Relations between Morphology and Physiology of *Aspergillus Oryzae*.

By Yoshio OTANI.

From the Zymomycological Institute, the Imperial College of
Agriculture, Tottori, Japan.

(Received Feb. 17, 1939.)

INTRODUCTION.

Aspergillus Oryzae is a fungus which has an important part in brewing saké, shōchū (Japanese spirits), soy sauce and miso (soy bean paste). In 1878, Ahlburg⁽¹⁾ gave it the name *Eurotium Oryzae*. Later Cohn⁽²⁾ revised the name to *Aspergillus Oryzae*, which is the current term used now. But at that time, the morphological description was very simple, and was not sufficient to show the characteristics of this species. Later, Büsgen⁽³⁾ made a more careful study of the subject and showed the characteristics of this organism more clearly. Wehmer⁽⁴⁾ isolated a strain of this fungus, through the pure culture method, and made a careful study of it. Thus the micro-biological characteristics were first shown in detail. Also, Klöcker and Shiönning⁽⁵⁾ found that this fungus produced sclerotia. Studies of the enzymes of this fungus have been made by Atkinson⁽⁶⁾; Kellner, Mori and Nagaoka⁽⁷⁾; Stone and Wreight.⁽⁸⁾ But a comparative study of the details of the morphological and physiological characteristics of the fungi, given in the studies made by these scholars, showed that there were a few points on which they disagreed. Takahashi⁽⁹⁾ took some strains which he had isolated from moyashi (koji-flora) and made comparative experiments with these, and showed clearly that this species could be classified into a number of varieties. Oshima⁽¹⁰⁾ found that from the enzymatic standpoint, the fungus could be divided into some groups and the groups classified according to the degree of enzymatic action in each. Recently, Sakaguchi⁽¹¹⁾ classified them according to their ability to produce metabolic products, such as alcohol and various acids. He⁽¹²⁾ also made a classification, according to the colour and form of the mycelia of the fungus. Thus we see that *Aspergillus Oryzae* is only one species, but has in it groups which have some different characteristics.

The author has collected from various places, numerous strains of this fungus, and made a study of their morphology and physiology, trying to get a clearer knowledge of the relation between the two, the results of which are given below.

METHODS OF EXPERIMENT AND OBSERVATION.

Morphological studies :— Various strains of *Aspergillus Oryzae*, were cultivated on koji-agar at the temperature of 30°C, for two whole days, and the height of the mycelia mass, the diameter of the conidium, the length and diameter of the sterigma, the diameter of globule, the length and diameter of the conidiophore, and the width of the hyphae were examined both with the naked eye and under the microscope.

Physiological studies :— A study was made of the starch-decomposing power, protein decomposing power, the acid producing quality and the colour which was shown. The essential points and the methods are as follows :—

To measure the starch-decomposing power the medium was made through Peffer-solution, which has no cane sugar in it (the solution has 1% of ammonium nitrate, 0.5% of potassium bi-phosphate, 0.25% magnesium sulphate, a trace of iron chloride). Soluble starch was added as a carbon source in the following ratio, *i. e.*, 0.7%, 1.4%, 2.2%, 3.2%, 5% and 8%, and in these media the numerous strains of the fungi were inoculated. By the use of the iodine method, the degree of starch digested was noted day by day. If the strain had great power to decompose starch, when put into the incubator for 3 days, at the temperature of 30°C, the starch in the 0.7% soluble starch medium was digested. In the medium which had 8% of starch, there was no strain which was powerful enough to change it. In the medium which had 5% starch, there were 7 strains which changed it to a blue-violet colour, through the reaction of iodine.

Koji-gelatine was used to measure the power to decompose protein. 20% of gelatine was put into the koji-extract, and allowed to congeal. And in this, as a medium, strains of *Aspergillus Oryzae* was inoculated, and was left at the room temperature (about 20°C) for from three to five weeks. The strain which had a strong protein decomposing power quickly liquefied the gelatine. Thus it could easily be seen that the medium divided itself into three parts, mycelia mass, the liquefied part, and the congealed part. When the liquefied part were seen to be large, it was clearly seen that the protein decomposing power was great.

To measure the acid producing power, koji-extract was used. In this medium the strains were inoculated, and were cultivated for fifty days. Finally 5 cc of the metabolic solution was taken and was neutralized with $N/10$ NaOH solution, using phenolphthalein as an indicator. Through the amount titrated, the degree of acidity was determined.

The power to produce pigment was determined as follows :— the strains were inoculated into koji-extract or koji-agar, and cultivated for five weeks, and with the naked eye, the degrees of the developing dark brown colour were noted.

RESULTS OF THE EXPERIMENT AND DISCUSSION.

Through these experiments, it was found that there are a great many minor differences in the various strains, both in their morphology and in their physiology. If these differences are minutely classified, from the enzymatic standpoint, both the starch-decomposer and the protein decomposer might be divided into two groups. In addition, from the metabolic standpoint, it may be divided into acid-producer and pigment producer. The author took five strains each from the strongest strains, and gave the averages of these strains in the following table :—

		Enzymatic classification		Metabolic classification	
		Starch-decomposer	Protein-decomposer	Acid-producer	Pigment-producer
Height of mycelia mass (mm)		3.06	1.90	3.1	1.94
Diameter of conidium (μ)		4.8~ 9.2	4.3~ 7.2	4.8~ 8.1	4.6~ 7.7
Sterigma	length (μ)	13.9~27.6	13.8~19.2	15.1~23.4	12.2~19.3
	diameter	4.3~ 5.4	3.8~ 5.2	4.5~ 6.0	4.8~ 6.0
Diameter of sterigma (μ)		22.0~39.9	16.1~26.2	19.2~26.9	18.7~27.0
Conidiophore	length (μ)	1.1~ 1.3	0.5~ 0.6	0.5~ 1.1	0.5~ 1.0
	diameter (μ)	8.3~13.2	5.8~11.0	7.0~12.9	6.1~10.6
Width of hyphae (μ)		3.5~ 9.0	4.9~ 8.9	3.8~11.2	3.3~10.8
Decomposition of starch		++±	±	+	−
Decomposition of protein		±	+++±	±	+
Production of acid (cc)		3.28	2.84	5.73	2.17
Pigment formation		±	±	+±	+++

Note:— − = 0, ± = few, + = the relative amount (indicated by the number of +).

As shown in the foregoing table, the greatest morphological difference shown was the height of the mycelia mass. The next greatest difference was in the diameter of the globules and in the length and diameter of the conidiophore. Comparing the morphological differences and the physiological differences, it was found that there was a direct relation between the starch-decomposing power and the protein-decomposing power, between the acid-producing power and pigment-producing power.

For example, there are many points of similarity, between the starch decomposer and the acid producer, in the following points :— the height of the mycelia, the diameter of the globules and the length and diameter of the conidiophore. Also the protein decomposer and the pigment producer were found to be very similar, in the morphology of the three parts mentioned above. From the physiological point of view, in the starch decomposer and in the acid producer, the starch-decomposing power and the acid producing power were found to be great. In the protein decomposer and in the pigment producer, the protein decomposing power was great. But in pigment production, the above mentioned relations did not hold good. So this side of the subject must be studied somewhat more thoroughly.

From the above facts, both the starch decomposer and the acid producer have an effect on carbohydrates. With regard to this, both the protein decomposer and the pigment producer seem to have an effect on the protein and its decomposition products. But the mechanism by which the dark brown colour appears in the *Aspergillus Oryzae* culture is not yet clearly understood, nor is the real construction of the pigment yet understood. So the author is not yet able to state any clear solution beyond that the above facts were noticed. In the various strains which the author used, there are some which seem to have both characters and there are some which seem to have a character between the two. But such variations seem to be a general characteristic of any micro-organism.

SUMMARY.

The author made a number of comparative experiments with regard to the morphology and physiology of various strains of *Aspergillus Oryzae.* The enzymatic characteristics may be divided into two, *i. e.,* starch decomposer and protein decomposer. There were two characteristics of the metabolic product, *i. e.,* acid producer and pigment producer. The morphology and physiology of the starch decomposer and the acid producer were very similar. Those of the protein decomposer and pigment producer were also very similar. Thus, we were able to place the starch decomposer and the acid producer in one group which has the power to act on carbohydrates. Both the protein decomposer and the pigment producer were classified in the other group which acts on protein and on its decomposition products. In addition, among the various strains, there were some which were between the two classes mentioned above, and there were some which had the characteristics of both groups.

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ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Retting of Vegetable Fibre Materials.

Part VIII. The Useful Anaerobes for the Bacterial Retting of Hemp Fibre Materials.

(pp. 207～212)

By Hideo KATAGIRI and Tosio NAKAHAMA.
(Agr. Chemical Laboratory, Kyoto Imperial University and Kanebo Yamasina Institute; Received Feb. 2, 1939.)

Nearly the same effective retting of hemp fibre materials was attained by the anaerobic process as was previously observed by us with the aerobic bacteria (see Part VI & VII), and ten strains of anaerobic bacteria were isolated from the retting vat.

When pure fermentations of hemp fibre materials were carried out with these ten strains of bacteria, one strain of bacillus and one strain of coccus were selected as the most useful organisms.

One of the useful anaerobes was classified as a new species as *Kurthia cannabis liquefaciens*, since the characteristics of the bacteria were found not to be the same as those of Kurthia zopfii Trevisan, in the liquefaction of gelatin and in the production of indole or the acid formation from sugars.

The other useful gram-positive anaerobe was found to reveal similar cultural characteristics to Micrococcus cremoris-viscosi Hammer and Cordes. However, some physiological natures were quite different, since slimy fermentation of milk was never observed, gelatin was liquefied in infundibuliform and indole was produced by the bacteria.

It was therefore concluded that this bacteria was also a new species and it was named *Diplococcus cannabis.*

Effects of certain Minerals on the Growth of Root Nodule Bacteria. (Part II.)

(pp. 213～224)

By K. KONISHI, T. TSUGE and A. KAWAMURA.
(Institute of Agr. Chem., Imp. University, Kyoto; Received Jan. 18, 1939.)

It was shown that most of the minor elements detectable in root nodules or roots exerted no oligodynamic effects on the growth of the organisms, but Mn and Cr did stimulate alfalfa bacteria on nitrate media, as well as Li lupine bacteria on yeast water media. A beneficial effect of ashes of water extracts from tops of several crops, when added to the culture, might be ascribed to their mineral constituents in special proportions. It suggested that increasing amounts of Na-sulfate and adding Ca-salt to the basic media were desirable in so far as the development of the organisms was concerned.

The Metabolic Products of Penicillium funiculosum Thom. (Part I.)

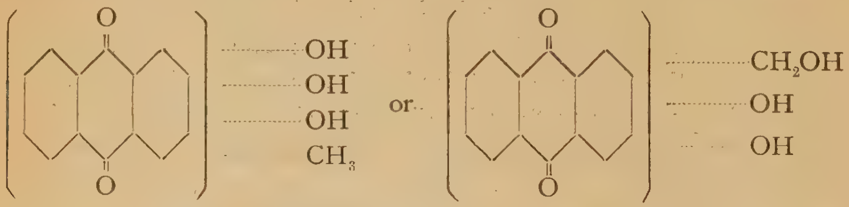
A Red Pigment, Funiculosin. (I).

(pp. 225～228)

By Hisanao Igaraci.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received Feb, 1,1938.)

A mould fungus, Penicillium funiculosum Thom, was cultivated in Koji-decoction. After abont twenty days, the mycelium was gathered, treated with dilute NaOH solution, and filtered. To the clear violet solution so obtained, an excess of dilute hydrochloric acid was added. The red colored matter, which had precipitated, was dried, and extracted with light petroleum ether. On evaporating the petroleum ether from extract, red crystalline pigment, which was named funiculosin, $C_{15}H_{10}O_5$, m. p. 218° was obtained. Funiculosin yielded triacetyl (m. p. 205°) and tribenzoyl (m. p. 277°) derivatives, showing that funiculosin has 3 hydroxyl groups. By dry distillation of funiculosin with zinc dust, anthracen was obtained. By the reduction of funiculosin in alcoholic solution, the red color faded, but the color reappeared by the contact with air. Hence it was assumed that funiculosin was a quinonoid. From the results above described, the following formula was proposed for funiculosin.



The Metabolic Products of Penicillium funiculosum Thom. (Part II.)

Organic Acids.

(pp. 229～230)

By Hisanao Igarasi.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received Feb. 1, 1938.)

Penicillium funiculosum Thom was cultured in Koji-decoction for 15～25 days. The mycelium was filtered off and the filtrate was evaporated to a small bulk, acidified with sulphuric acid and extracted with ether. After the evaporation of ether from extract, fine crystals of an organic acid which was identified with malonic acid, were isolated. When the synthetic medium, containing glucose and ammonium sulphate, was used in place of Koji-decoction, malonic acid together with succinic acid were obtained after 15 day's cultivation, but succinic acid only was obtained after 30 day's cultivation, and oxalic acid after 45 day's cultivation.

The Constituents of the crude Lecithin of Soyabean.

(pp. 231～234)

By Koji Okano and Iwao Beppu.

(The Central Laboratory of the South Manchurian Railway Co., Received Feb. 6, 1939.)

Studies on Soyabean Oil-foots.

(III) Isolation of Flavin.

(IV) Manufacture of the Lactation-promoting Medicine and Tests of its Effects.

(pp. 235～242)

By Koji Okano, Iwao Beppu and Masatoshi Ozu.

(The Central Laboratory of the South Manchurian Railway Co., Received Feb. 6, 1939.)

Chemical Studies of Green Tea. 3.

On Pectin and on the Action of Pectinase on it.

(pp. 243～249)

By Saichi Machida and Toshio Tashiro.

(Laboratory of Shinsei-Shokuhin Co. Ltd., Received Jan. 24, 1939.)

Decomposition Products of Substance containing Uronic Acid in Autoclave (IV).

Formation Mechanism of Alginetin.

(pp. 250～252)

By Kiyosi Aso.

(Tokyo Imperial University, Received Feb. 13, 1939.)

Studies on the Vegetable Tannins in Formosa. Part II.

Isolation of Tannin and its Diffusion Velocity into Gelatin-gel and Absorption Amount by Hide Powder.

(pp. 253～256)

By Yasuyosi Osima and Yôsuke Kaneko.

(Agricultural Chemical Department, Taihoku Imp. Univ, Taiwan, Japan,
Received Feb. 13, 1939.)

1) The raw materials reported in Part I were pulverized and repeatedly

extrated with warm water and the extract was saturated with salts and precipitate produced thereby was filtered off. The precipitate was dried in vacuo and extracted with aceton. From the extract, aceton was evaporated in vacuo and the residue was dissolved in warm ethyl acetate. Adding of chloroform into the ethyl acetate solution, tannin (A) was precipitated and there were dried in vacuo.

The filtrate of saturation with salt was shaken with ethyl acetate repeatedly and ethyl acetate extract was concentrated in vacuo and added an excess of chloroform. The precipitate of tannin (B) thus formed was dried in vacuo.

According to the original raw materials, tannins (A) and (B) was isolated respectively.

The results of analysis of isolated tannins were as follows:

Tannin	Soluble matter	Tannin	pH of 1% sol.
Acacia decurrens (A)	87.4	83.7	3.9
Acacia confusa (B)	95.8	92.7	4.0
Areca catechu (B)	97.1	97.1	3.9
Thea sinensis (B)	95.3	94.2	3.3
Dioscorea cirrhosa (B)	93.0	90.1	3.9
Dioscorea Matsudai (B)	93.1	90.2	3.9
	95.6	93.4	3.2
	95.7	94.3	2.3
Therminalia chebula (A, B)	95.3	92.4	2.8
Gallotannin	91.1	84.4	3.3
d-catechin (m p 217)	101.0	90.1	4.2

The qualitative reaction of isolated tannins were entirely equal with the extract of raw materials and then the tannins were the main tannin in the raw materials.

2) Diffusion velocity into gelatin-gel. The hot solution contents 10% of gelatin and 0.1% of ferric chloride was coagulated in the glass tube of dia. 0.4 cm and length 10 cm. Take 15 cc of 1% of the tannin in the buffer solution of pH 4, 6, 8 respectively and into it the above glass tube was inserted for 72 hours at 25°C and the black colour tinted by the diffusion of tannin were measured.

The diffusion velocity was various according to the kind of tannin and in general pyrogallol tannin was fast and was faster in alkaline side.

Tannin	After 24 h.			After 72 h.		
	pH 4	6	8	4	6	8
Gallotannin	1	5	10	2	7	17
Acacia decurrens	0	0	1	0	0	1
Acasia confusa	0	1	3	0	2	5
Areca catechu	0	0	4	0	0	4
Thea sinensis	0	4	6	1	5	10
Dioscorea cirrhosa	0	0	1	0	0	2
Terminalia chebula	7	12	16	11	15	23

3) Absorption amount of tannin by hide powder. Take 15 cc of 1% solution of the tannin in buffer solution of pH 2, 3, 4, 5, 6, 7, 8 and into it 0.1 g of hide powders were added and settled for 24 hours at 25°C. The hide powders were filtered off and washed until the reaction of tannin has disappeared and dried at 95°～100° and weighed. The amount of tannin absorped by the hide powder were as follows.

Tannin	pH 2	3	4	5	6	7	8
Acacia decurrens	33.0	22.6	13.6	17.9	23.3	23.5	23.9
Acacia confusa	14.2	15.6	18.9	16.7	12.6	16.7	13.4
Areca catechu	31.5	22.3	14.6	15.2	16.7	18.9	21.5
Thea sinensis	12.6	13.3	15.3	14.6	14.7	16.7	12.0
Dioscorea cirrhosa	27.0	22.1	17.5	18.6	19.5	21.2	22.7
Terminalia chebula	7.0	4.8	2.5	1.6	0.5	0.5	0.4
Gallotannin	35.6	25.8	17.6	14.3	17.5	21.6	27.0

The amount of tannin absorped by the hide powder was various according to the kind of tannin and in the case of Acacia decurrens, Areca catechu, Dioscorea and gallotannin the minimum point has existed at pH 4～5 and the maximum points were 2 and 8. In the case of Acacia confusa and tea, the minimum and maximum points has removed to neutral point.

Biochemical Studies on "Bakanae" Fungus of the Rice. Part II.

Isolation of "Gibberellin", the Active Principle which makes the Rice Seedlings grow slenderly.

(pp. 257～266)

By Teijiro YABUTA and Takeshi HAYASHI.

(Imperial Agricultural Station; Received Feb. 6, 1939.)

Kurosawa has reported that the seedlings grown in the sterilized filtrate of the culture solution of the "Bakanae" fungus show similar abnormal elongation to the so called "Bakanae", i.e. remarkably slender rice seedlings infected by the fungus naturally found in the rice nursery; hence he has suggested that the Bakanae phenomenon is caused by a toxic substance formed by the causative fungus.

The author attempted the isolation of the stimulus substance in crystalline state in order to make clear its chemical nature and mechanism of physiological action on the plant.

The successful procedure for the isolation of the active principle is as follows.

The most active strain among the Bakanae fungi is selected and cultured at 25°C for 30 days in the medium consisting of glycerine (3 g), ammonium chloride (3 g), acid potassium phosphate (3 g) and tap water (1000 cc), the pH of the solution being adjusted to 3.2 with hydrochloric acid. After that period the culture medium is filtered and sufficient amount of active carbon added to the filtrate with mechanical stirring; whereby the active principle is almost perfectly adsorbed. It is set aside overnight, the supernatant clear liquid then siphoned off, the sediment filtered by suction, washed with water and well drained on a Buchner's funnel. The adsorbate is now eluated with methanol-ammonia in the room temperature. To the eluate, after evaporation of the solvent under diminished pressure, basic lead acetate is added and the resulting precipitate is filtered off. The filtrate is treated with hydrogen sulphide and the filtrate from lead sulphide is reduced in vacuo to a small volume and extracted with ether in a soxhlet apparatus after addition of sodium bicarbonate; whereby basic, neutral and phenolic contaminations soluble in ether are removed. The extraction with ether is continued after acidification with sulphuric acid. On evaporating the ether extract and drying the residual syrup in vacuo over sulphuric acid, an almost colorless amorphous powder is obtained.

This substance thus prepared is effective for rice seedlings in quite minute amonuts, e. g. even in 0.00002% solution.

The authors propose the name "Gibberellin" for the active principle.

Gibberellin is easily soluble in alcohol, acetone and aqueous alkaline hydroxide or carbonate; fairly soluble in water and ether; difficultly so in chloroform, benzene and xylene; but insoluble in carbon disulphide, carbon tetrachloride and petroleum ether.

Gibberellin is quite labile in aqueous alkaline solution, losing its potency even in ordinary temperature. In acidic solution, however, it is rather stable.

Recently T. Yabuta and Y. Sumiki have succeeded in resolving the crude amorphous gibberellin into two different crystalline substances, both active for plants, by means of careful fractional crystallisation from ligroin mixed with ethyl-acetate or ethanol; one occurs as long prisms, m. p. 194～6° [C% = 75.16 H% = 7.60 M. W. = 250 (by Rast)] for which the name Gibberellin A is provisionally proposed and the other occurs as short prisms, m. p. 245～6° (decomp) [C% = 65.06, H% = 7.12, M. W. = 386 (by Rast), 357 (by titration as monocarboxylic acid) $[\alpha]_D = +36.13°$ (in methanol)] which has been termed Gibberellin B.

Studies on the Action of Arginase on Canavanin and Arginine III.

On the constitution of guanidine derivatives as the substrate for arginase.

(pp. 267～279)

By Natsunosuke KITAGAWA.

(Biochemical Laboratory, Department of Agriculture, Kyushu Imperial University, Fukuoka; Received Feb, 3, 1939.)

On the Constituents of the Extract of Bamboo Sprouts.

(pp. 280∼282)

By Teizo TAKAHASHI and Yoshinao YOKOYAMA.

(Agricultural College Gifu, Japan, Received Jan. 23, 1939.)

The following constituents were isolated from the water extract of bamboo sprouts (Phyllostachys mitis).

Oxalic acid	0.0533% of the material	0.1006% of the edible portion
K-tetraoxalate	0.0019	0.0037
Betaine	0.0097	0.0183
Tyrosine	0.0386	0.0729

On the Constitution of *α*-Sorigenin.

(pp. 283∼290)

By Zirô NIKUNI.

(Agr. Chem. Laboratory, Tokyo Imp. Univ., Received Feb. 23, 1939.)

α-Sorigenin was obtained as faintly yellowish fine needles from the bark of Kuroumemodoki (Rhamnus japonica, Max.). (Bull. Agr. Chem. Soc. of Japan: **14**, 25 (1938); **15**, 15 (1939)

It is soluble in alcohol, methanol and acetone but insoluble in water. Dilute alkaline solution dissolves it and the sunlight turns it to violet. It melts at 227∼229° and its experimental formula is $C_{13}H_{10}O_5$.

The constitution of this compound was determined as follows:

A. Determination of five oxygen atoms.

1) Two free hydroxyl-groups.

The alcoholic solution of *α*-sorigenin turns to deep greenish brown by ferric chloride solution. It takes two acetyl-groups by acetylation with acetic anhydride and sodium-acetate, and takes also two methyl-groups by methylation with diazomethane. These derivatives have no colour reaction with ferric chloride solution.

2) One methoxyl-group by Zeisel's method.

3) Lactone ring.

By dilute caustic alkaline solution *α*-sorigenin and *α*-sorinin take one molecule of water and then precipitate as their monoalkaline salts. And these salts return easily to their original compounds by acid. Therefore the existence of γ or δ lactone ring was determined.

The new hydroxyl-group, produced by the breakdown of lactone ring, has no colour reaction with ferric chloride solution, and takes no methyl-group by the methylation with diazomethane. So it must be an alcoholic hydroxyl-group.

4) Carbonyl, methylendioxide, free carboxyl and carboxylmethylester-groups do not exist.

5) The existence of the following four compounds was tested by their characteristic colour reactions but they were all negative.

(a) 4′-Hydroxyflavone.
(b) Hydroxy-methyl-anthraquinone.
(c) Diphenylen-dioxide.
(d) Coumarin.

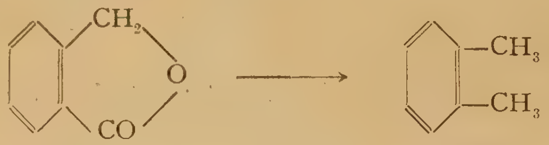
So the five oxygen atoms were all determined two as free hydroxyl, one as methoxyl, two as lactone ring.

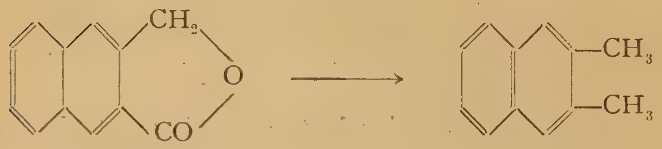
B. Determination of naphthalene nucleus.

When heated with alkali α-sorigenin and their derivatives give out a naphthalene-like smell.

By the zinc dust distillation in hydrogen gas stream, diacetyl-α-sorigenin gave a small quantity of yellowish oil with characteristic naphthalene-like smell. This oil separates from itself colourless plates on cooling below −10°. On recrystallization from dilute alcohol the colourless crystals melt at 90∼96°. Its absorption spectrum resembles that of naphthalene, demonstrating that the crystals may be a derivative of naphthalene.

The analytical result of this crystal coincides very well with that of dimethyl-naphthalene $C_{12}H_{12}$. Among ten isomers of dimethyl-naphthalene, 2, 3; 2, 8; 1, 5; 2, 6 and 2, 7-dimethyl-naphthalenes were obtained as crystals, but in these isomers the probable form is only 2, 3-dimethyl-naphthalene (m p. 104°) from the positions of side chains.

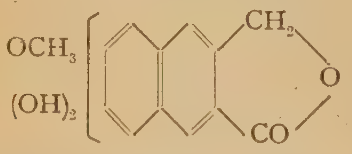

Phthalide
o-Xylol


Lactone of 3-hydroxymethyl-β-naphthoic acid
2, 3-Dimethyl-naphthalene

From the fact that the phthalide gave o-xylol by zinc dust distillation, it is very probable that the lactone of 3-hydroxymethyl-β-naphthoic acid may give 2, 3-dimethyl-naphthalene by the same treatment.

From these experimental results and discussions the author concludes that α-sorigenin may be the lactone of monomethoxy-dihydroxy-3-hydroxymethyl-β-naphthoic acid.

Methoxyl and hydroxyl groups may be combined with naphthalene nucleus, but their positions are not yet decided.


α-Sorigenin
=Lactone of monomethoxy-dihydroxy-3-hydroxymethyl-β-naphthoic acid

The author expresses his sincere thanks to Prof. B. Suzuki for his kind guidance throughout this work.

The Influence of Monochromatic Lights on the Action of Enzymes.

[Report XII.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 1.) Yeast Saccharase.

(pp. 291~299)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya, Received Jan. 31, 1939.)

In order to investigate the influence of ultra-violet rays on the action of the saccharase in the yeast, the author has employed "Vim Ray" blue lamp. Enzyme solution was prepared as follows: After the liquefaction was completed by the addition of 10% toluene into 225 g of "Oriental" pressed yeast the same weight water was added and the yeast was allowed to autolyze at room temperature for 7 days. The precipitation obtained by adding an equal volume of alcohol to the autolyzed liquid was extracted by 20% alcohol. To the extract was then added alcohol and the second precipitate was extracted with 100 cc of water.

The author has added 1 cc of the enzyme solution into the test tubes containing 4 cc of 20% saccharose dissolved in 1% primary sodium phosphate solution and the pH of the solution adjusted to 4.24.

The test tubes were placed within the tin boxes, at the front windows of which were placed ultra-violet-close-filter and ultra-violet-pass-filter. The filters of white and black passing respectively ultra-violet and visible or no rays were also applied as controls. The boxes containing the test tubes were incubated at 40°C and the door opened, and lighted by the "Vim Ray" blue lamp through the layer of copper sulphate solution and filters. The transmission wave langths of each filter were as follows: white······3500~5500 I. Å., ultra-violet-close······ 4100~5500 I. Å., ultra violet-pass······3565~3984 I. Å., black······none.

The test tubes were taken out from each box at the intervals of 30, 60, 90 and 120 minutes after the incubation, and the each tube 2 cc of saturated Na-carbonate solution were added and the contents diluted to 250 cc with water and then the determination of invert sugar contents were made by Bertrand's method.

In this experiment, the action of the yeast saccharase was found to be inhibited by the ultra-violet rays and promoted by the visible rays. The action of the enzyme was promoted at first under the influence of the rays containing both ultra-violet and visible, but the action of the enzyme was finally inhibited.

[Report XIII.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 2.) Yeast Amylase.

In order to investigate the influence of ultra-violet rays on the action of the amylase in the yeast, the author has employed "Vim Ray" blue lamp. Enzyme solution was prepared by the autolysis for one day at room temperature and then for overnight in an ice box after adding 20 cc of toluene and 500 cc of water into

200 g of "Oriental" yeast.

The author has added 10 cc of the enzyme solution and 10 cc of phosphate buffer solution to the test tubes containing 10 cc of 1% soluble starch solution, and the pH of the solution adjusted to 5.1. The tubes were placed within tin boxes, at the front windows of which ultra-violet tight filter, ultra-violet pass filter, white filter and black filter were placed. The boxes containing the test tubes were incubated at 40°C and lighted by "Vim Ray" the lamp through the layer of copper sulphate solution and filters. As to the qualities of the filters see the suthor's previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 8, 16 and 24 hours after the incubation and then the end product of the hydrolysis by the enzyme was determined by Bertrand's method.

In thir experiment, the action of the yeast amylase was found to be inhibited under the influence of ultra-violet rays and promoted by the visible rays. The action of the enzyme was also promoted by the rays containing both ultra-violet and visible rays.

Studien über Sericin. V. Mitteilung.

(ss. 300～304)

Von Takeo Ito und Kozo Komori.

(Aus dem chem. Laboratorium der Seidenbau-Hochschule, Kyoto, eingegangen am 15. Feb. 1939.)

In der vorliegenden Untersuchung wurden die bei dem Erhitzen im Wasser auftretenden Veränderungen der Sericinfraktionen weiter verfolgt. Sericin A wurde durch kurzdauernde Erhitzung bei 105° im Wasser gelöst. Je 50 ccm von dieser Lösung wurden unter den in Tab. 1 angegebenen Bedingungen erhitzt, nach dem Abkühlen mittels eines Azetat-Puffers gepuffert und sodann auf 75 ccm aufgefüllt. Hierauf hat man pH, Viskosität und das durch 50%-igen Alkohol koagulierbare Sericin-N bestimmt. Die Resultate sind in Tab. 1 zusammengestellt.

Tabelle 1. Die durch Erhitzung verursachte Verminderung der Viskosität und der Alkoholkoagulierbarkeit von Sercin-A-Lösung.

Erhitzungs-		Konzentration (mg N/100 ccm)	pH	Relative Viskosität (25°)	Durch 50%-igen Alkohol koagulierbarer Sericin-N (% gegen Gesamt-N)
Temperatur	Dauer				
——	——	100.3	3.87	1.093	85.70
100°	1 Stde.	99.5	3.86	1.086	80.19
110°	0.5 〃	99.8	3.89	1.067	58.82
〃	1 〃	100.4	3.89	1.061	45.94
〃	2 Stdn.	100.6	3.93	1.058	26.51
120°	1 Stde.	100.3	3.88	1.061	28.47

Es ist zu ersehen, daß durch Erhitzung die Viskosität als auch die Alkohol-Koagulierbarkeit der Sericin-A-Lösung vermindert wird, und zwar die letztere in starkem Maße.

Auf Grund dieser Tatsache, zumal wenn man die damit gleichzeitig auftretende Ammoniak-Abspaltung, welche in einer früheren Arbeit festgestellt worden ist, in Betracht zieht, so ist es leicht annehmbar, daß sich die Micelle von Sericin A bei höheren Temperaturen irreversibel desaggregiere und dadurch verkleinere.

Versuche mit Sericin B wurden folgendermaßen durchgeführt: Verschiedene Mengen von Sericin B wurden je mit 50 ccm Wasser eine Stunde lang bei einer gegebenen Temperatur erhitzt. Dabei ging Sericin B stets in teilweise Lösung über. Die Lösung wurde noch gelinde warm samt dem ungelösten Teil in einen Meßkolben eingegossen, nach dem Abkühlen mit 10 ccm von 0.2 molarer Azetatpufferlösung versetzt, dann auf 100 ccm aufgefüllt und unter Toluol-Zusatz bei 25° über Nacht stehen gelassen. Mit dem aliquoten Teil wurde der aufgelöste Stickstoff nach Kjeldahl bestimmt.

Beistehende Abb. 1 gibt die erhaltenen Resultate graphisch wieder, wobei auf der Abszisse die beim Versuche angewandten Mengen von Sericin B in Gramm, und auf der Ordinate die Mengen vom aufgelösten Stickstoff in Milligramm aufgetragen wurden. Sie zeigt, daß Sericin B annähernd proportional mit dessen Menge, welche beim Versuche angewandt wird, in Lösung geht. Analoge Tatsachen sind bereits vorlängst von Hardy und Mellanby (1905) gefunden worden. Nach ihren Versuchen ist nämlich die bei einer bestimmten Salzkonzentration gelöste Globulinmenge mit derjenigen Globulinaufschlämmungsmenge, welche bei dem Lösungsversuche in Arbeit genommen wurde, annähernd proportional. Durch umfassende Versuche konnte Sörensen (Koll. Z. 53, 110 (1930) nicht nur die Beobachtung der genannten Forscher bestätigen, sondern er konnte noch beweisen, daß eine solche partielle Lösung oder eine partielle Fällung vom Globulin eine Fraktionierung des Globulins herbeiführt. Nach ihm kann diese Tatsache dadurch einfach erklärt werden, daß man sich die Globulin als leicht dissozierbare Verbindungen, nicht aber als Mischungen zweier oder mehrerer Fraktionen vorstellt. Er nimmt weiter an, daß sowohl der undissozierte Globulinkomplex, als auch die daraus gebildeten Dissoziationsprodukte höchst verschieden in Salzlösung löslich seien. Wird ein solcher Globulinkomplex mit einer Salzlösung, die zur vollständigen Lösung des Globulins nicht hinlänglich konzentriert ist, behandelt, so werden nur die am leichtesten löslichen Dissoziationsprodukte in Lösung gehen. Bei Anwendung von größeren Globulinmengen, werden sich dementsprechend größere Mengen von den löslichen Dissoziationsprodukten auflösen.

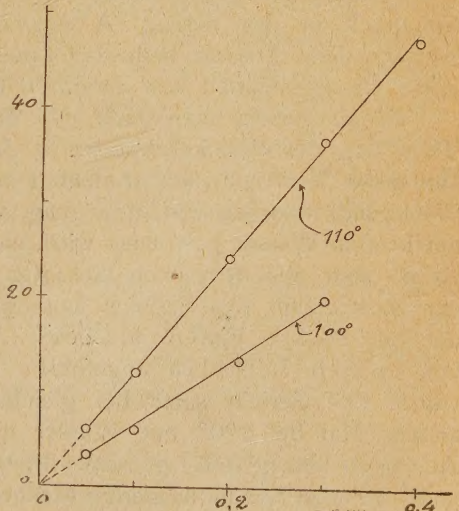


Abb. 1.

Auf diese Weise erhält man eine einfache Erklärung für das angeführte sonderbare Auflösungsverhalten des Globulins.

Die Auflösung von Sericin B in heißem Wasser muss indessen etwas anders aufgefasst werden, denn hier handelt es sich zweifellos um einen irreversibelen Vorgang, welcher von dem reversibelen Vorgang wie im Falle des Globulins scharf getrennt gehalten werden muß, was auch schon von Sörensen (loc. cit.) selbst nachdrücklich betont worden ist.

Wahrscheinlich wird sich Sericin B bei der Behandlung mit Wasser bei höheren Temperaturen irreversibel desaggregieren (bzw. spalten), und eine oder mehrere von den Desaggregationsprodukten werden in Lösung gehen. Für diese Deutung spricht auch, daß der dabei ungelöst zurückbleibende Anteil anders zusammengesetzt ist als das zuerst zum Versuche angewandte Sericin B, wie aus der folgenden Tabelle zu ersehen ist.

Tabelle 2.

	Gehalt an Kohlenhydrat in mg pro 100 mg Sericin-N		Verteilung vom Stickstoff				
	Zucker (als Glukose berechnet)	Aminozucker (als Glukosamin berechnet)	Ammoniak-N	Melanin-N	Diaminosäure-N	Monoaminosäure-N	Summe
Sericin A	1.8	1.9	12.14	0.17	16.56	70.10	99.58
Sericin A_1	2.4	3.5	10.89	0.85	17.47	70.53	99.88
Sericin B	7.3	7.5	10.22	0.90	18.20	69.19	99.65
Sericin B_1	15.9	13.2	9.34	1.16	20.01	68.96	99.47

In Tab. 2 bedeutet Sericin A_1 den in heißem Wasser aufgelösten und nachher durch Alkohol-Zusatz (50%) ausgefallenen Anteil von Sericin B, und Sericin B_1 denjenigen Anteil, welcher ungelöst zurückblieb.

Es wird oft ausgesprochen, daß sich das Sericin B bei höheren Temperaturen allmählich in das Sericin A umwandele. Unserer Erfahrung gemäß, dürfte indessen diese Aussage nicht dahin ausgehen, daß, wie oben erwähnt, durch irreversibele Desaggregation von Sericin B ein wasserlöslicher Anteil entsteht.

Ferner spricht man auch von der Wichtigkeit der Kenntnisse bezüglich der Verteilung der Sericinfraktionen in der Kokonschicht für die „Filature" Chemie. Um diese Verteilung experimentell zu ermitteln, verfährt man gewöhnlich in der Weise, daß man den Sericin-Auszug, welcher aus Kokonschicht durch Behandlung mit heißem Wasser gewonnen wird, nach einem oder anderem Verfahren analysiert. Es ist aber aus dem oben Gesagten leicht ersichtlich, daß bei den Bedingungen der Ausziehung des Sericins namentlich die Temperatur auf die Analysierungsresultate starken Einfluß ausüben. Als Beispiel hierfür seien die Resultate eines von unseren Versuchen angeführt: Aus der Kokonschicht von derselben Art wurde das Sericin sonst bei gleichen Bedingungen einmal bei 110°, und das andere Mal bei 120° mit Wasser ausgezogen. Der gesamte N in den beiden Auszügen betrug 3.677 g resp. 3.798 g pro 100 g Kokonschicht. Sie wurden nach der von uns modifizierten Mosherschen Methode analysiert. Auf diese Weise ergab sich der Zahlenwert für Sericin-B-N im ersten Falle zu 60.09% und im zweiten Falle zu 27.42% vom gesamten N, also besteht ein ausserordentlich großer Unterschied zwischen den beiden Resultaten.